CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to: Commissioner for Patents; Mail Stop Amendment; P.O. Box 1450; Alexandria, VA 22313-1450

Rebecca A. Bellas
(Type or print name of person mailing paper)

May 24, 2005 Rebecca A. Bellas

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application

Applicant:	Horres, et al.	Art Unit:	3738
Serial No.:	09/914,241	Examiner:	Thomas C. Barrett
Filed:	August 24, 2001		

Title: HEMOCOMPATIBLE SURFACES AND METHOD FOR PRODUCING SAME

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Erika Hoffmann, declare and say as follows:

I hold a Bachelor's degree from the Technical University of Aachen, Germany, a Master's degree from the Technical University of Aachen, Germany, and in the near

future a Ph.D. degree from the Technical University of Aachen, Germany. I have worked in biotechnology research for over five years and have extensive experience in the field.

I am the inventor of the invention described and claimed in the above-identified patent application and, therefore, I am thoroughly familiar with the subject matter of the invention.

Certain claims of the above-identified patent application stand rejected for lacking novelty over U.S. Patent 6,090,995.

Experimental tests were conducted by me in order to demonstrate that the claimed hemocompatible surface is indeed novel and thus different from the surface modifying composition described in U.S. Patent 6,090,995. U.S. Patent 6,090,995 describes the incorporation of biological materials into the surface modifier composition of a device, the biological materials including heparin or heparin sulfate proteoglycan.

1. Introduction

These experiments were performed to demonstrate the differences of a constituent obtained from an outer layer of a blood cell or an outer layer of a mesothelial cell versus like named constituents that are not obtained from a blood cell or a mesothelial cell. Accordingly, the hemocompatible potential of the substances isolated from erythrocytes and mesothelial cell glycocalices was investigated in relation to materials not obtained from a blood cell or a mesothelial cell.

a. Experimental Organization

The substances were covalently immobilized on cellulose membranes and then used for in vitro blood tests in order to determine blood platelet adhesion on the membrane surface. Measurement of platelet adhesion is a well-established method to

test the thrombogenicity of foreign surfaces. A high number of deposited thrombocytes means low hemocompatibility; low or no deposition of thrombocytes means better or high hemocompatibility.

The results were compared to the results from several commercially available glycosaminoglycans of different origin treated by the same procedure. The following eight commercially available glycosaminoglycans were used in the trials:

Materials <u>not obtained</u> from outer layers of erythrocytes and/or mesothelial cells (Commercially available glycosaminoglycans)

Chondroitin sulfate isolated from shark and whale cartilage (Sigma)	= ChS (shark)
Chondroitin sulfate from swine blood plasma	= ChS (swine)
Keratan sulfate from bovine cornea (gift)	= KS
Dermatan sulfate from swine chord (Sigma)	= DeS
Heparine from swine intestinal mucosa (Serva)	= Hep
Heparan sulphate from swine intestinal mucosa (Sigma)	= HS
Hyaluronic acid from human umbilical cord (Sigma)	= HA

The applicant provided the following materials to compare hemocompatibility:

Materials obtained from outer layers of erythrocytes and/or mesothelial cells (Claimed materials)

Endothelial cell surface heparansulfate (ESHS) from bovine aortae (produced by the Applicant)	= ESHS
Erythrocyte glycolcalix	= EryGlyco
Mesothelial glycocalix	= MesoGlyco

2. Experimental Procedure

a. Functionalization of Cellulose-Membranes for the Coupling Reaction:

The membranes were kept in a stirred mixture of ethanol/water (1:1) for 30 minutes followed by exposure to a 2% solution of 3-amino-propyl-triethoxysilane in ethanol/water (1:1) over night at 45°C. The functionalized membranes were rinsed with distilled water for 30 minutes, stored in ethanol/water (1:1) for 30 minutes at 45°C, and then washed thoroughly with water.

The 3-amino-propyl-triethoxysilane-cellulose membranes were deposited in 0.1 M 2-(N-morpholino)-ethanesulfonic acid buffer having pH 4.75. After cooling to 4°C, a solution of 0.1 % adipinic acid was added and N-cyclohexyl-N´-2-(morpholinoethyl)-carbodiimide-methyl-p-toluenesulfonate was dissolved in the buffer solution in several portions during 6 hours, until reaching a concentration of 2%. The next day, the membranes were washed as follows: first with buffer solution, ice-cooled water, 4 M NaCl and then again extensively with water.

b. Immobilization procedure:

The aminated and carboxylated membranes were immersed in a 4°C cold solution of 0.1% cyclohexyl-N´-2-(morpholinoethyl)-carbodiimide-methyl-p-toluolsulfonate in 0.1 M 2-(N-morpholino)-ethanesulfonic acid buffer at pH 4.75 and stirred for 30 minutes. After dipping in ice-cooled water for 60 seconds, they were stirred in the 0.1M 2-(N-morpholino)-ethanesulfonic acid buffer at pH 4.75 containing the substance in a concentration of 1 mg/ml buffer solution for 18h at 4°C. The next day, the membranes were washed with buffer solution, ice-cooled water, 4 M NaCl and again extensively with water.

c. Platelet adhesion test:

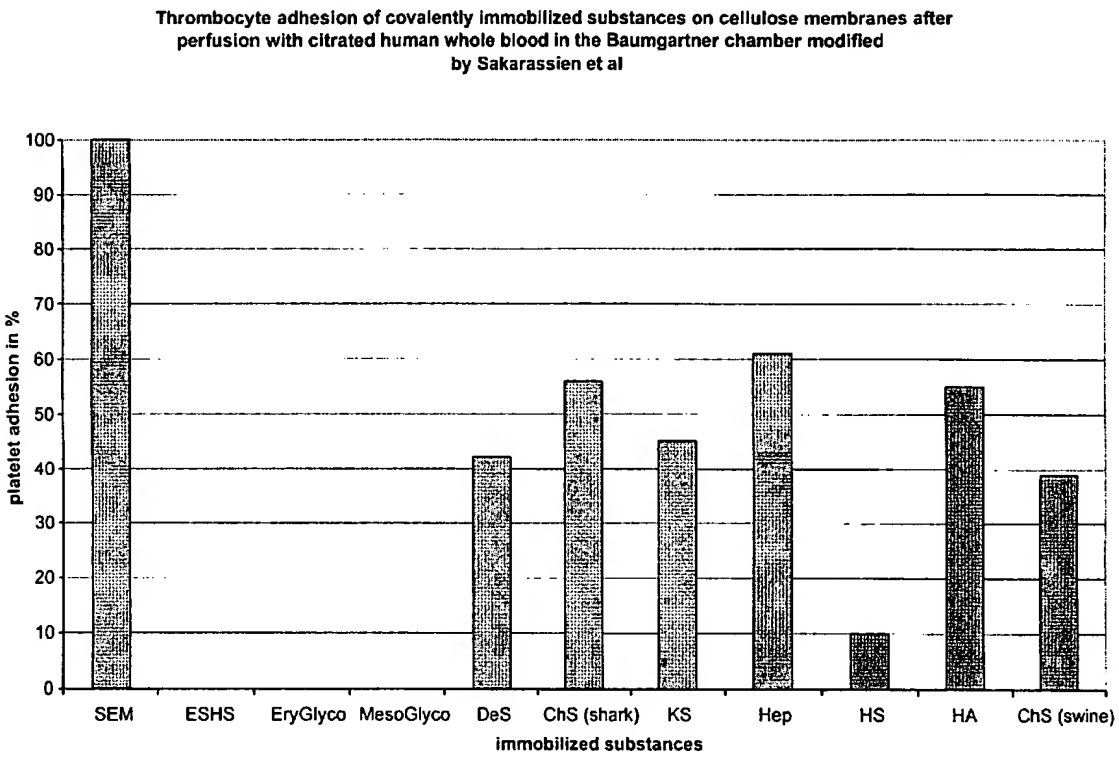
The membranes were perfused at laminar flow and shear rates of 1050 s^{-1} with citrated human whole blood in a Baumgartner perfusion chamber modified by Sakariassen et al (See J. Lab. Clin. Med. 1983, *102*, 522) for 10 minutes. Highly thrombogenic subendothelial matrix (SEM) was perfused as reference.

After fixing the adhering thrombocytes onto the membranes with glutardialdehyde, they were stained with eosin methylene blue solution (May-Grünwald solution and Giemsa solution obtained from Merck Diagnostica). The membranes were analyzed with a light microscope at 400 fold magnification. The thrombocyte adhesion is expressed as percentage platelet coverage in reference to the subendothelial matrix (SEM), given as 100%.

3. Results

Results are shown in the table below, and graphically on the next page. Commercial materials are in plain text, and **applicant's materials are in bold text.**

Material	% coverage
Chondroitin sulfate from shark/whale cartilage (Sigma) (ChS (shark))	- 56%
Chondroitin sulfate from swine blood plasma (ChS (swine))	- 39%
Keratan sulfate from bovine cornea (KS)	- 45%
Dermatan sulfate from swine chord (DeS)	- 42%
Heparine from swine intestinal mucosa (Serva) (Hep)	- 61%
Hyaluronic acid from human umbilical cord (Sigma) (HA)	- 55%
Heparan sulphate from swine intestinal mucosa (HS)	- 10%
Endothelial cell surface heparansulfate from bovine aortae (ESHS)	**- ~0%**
Erythrocyte glycocalix (EryGlyco)	**- ~0%**
Mesothelial cell glycocalix (MesoGlyco)	**- ~0%**



4. Discussion

The platelet adhesion results for the components of mesothelial cell glycocalix (MesoGlyco) and erythrocyte glycocalix (EryGlyco) are in the same range with the non-thrombogenic and highly hemocompatible endothelial cell surface heparansulfate (ESHS). The components of mesothelial cell glycocalix (MesoGlyco) and erythrocyte glycocalix (EryGlyco) as well as the endothelial cell surface heparansulfate (ESHS) are compounds which come in direct contact with blood and are required for the device surface to be hemocompatible. Consequently, the compounds can be used in the coating procedures in order to obtain the hemocompatible surfaces claimed in this invention.

The heparan sulfate from swine intestinal mucosa (HS) is not as hemocompatible as the components of mesothelial cell glycocalix (MesoGlyco) and erythrocyte glycocalix (EryGlyco). The heparan sulphate from swine intestinal mucosa (HS) shows moderate hemocompatibility (10%). However, in order to obtain the *highly* hemocompatible surfaces of the subject invention, the moderate hemocompatibility of the heparan sulfate from swine intestinal mucosa (HS) is insufficient. Consequently, heparan sulfate isolated from swine intestinal mucosa (HS) is not one of the substances encompassed within the claims of the invention.

All of the other commercially available glycosaminoglycans exhibit insufficient hemocompatibility. The figure shows clearly that use of these compounds does not render a surface sufficiently hemocompatible.

The present test results prove that of the broad group of glycosaminoglycans, only a few are able to solve the problem addressed by the subject invention, namely to make artificial surfaces hemocompatible. In this connection, these few compounds are the components of mesothelial cell glycocalix (MesoGlyco) and erythrocyte glycocalix (EryGlyco) as disclosed in the present invention.

I, Erika Hoffmann, hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued therein.

E. Hoffmann

Erika Hoffmann, Ph.D.

20.05.2005

Date